Anbio Rapid COVID-19 Antigen Test (Colloidal Gold) / Nasal Swab Clinical Trial Analysis Report

Report No:	Anbio-NS-20210305
Project Name:	Anbio Rapid COVID-19 Antigen Test (Colloidal Gold) / Nasal Swab Clinical Trial Analysis
Type of Clinical Trial:	Clinical Trial Analysis Validation
Clinical Trial Institution:	PacGenomics Clinical Genetics laboratory
Address:	28222 Agoura Road, Suite 200/201, Agoura Hills, CA 91301, USA
Principal Investigator:	Dr. Hua Li
Tel:	+1 818-597-1938
Email:	shuanghou@pacgenomics.com
Collection Site:	Hollywood Hills Women's Medical Group
Address:	9201 W Sunset Blvd, Suite 401, West Hollywood, CA 90069
Principal Investigator:	Dr. Karen Lapesarde
Tel:	+1 818-597-1938
Email:	labsite@hhwmg.com
Manufacturer:	Anbio (Xiamen) Biotechnology Co., Ltd.
Address:	No.2016, Wengjiao West Road, Xinyang Street, Haicang District, Xiamen, Fujian, China
Tel	+86 592 6312 399
Email:	info@anbio.com
Start Date of Clinical Trial:	02/26/2021
Completion Date of Clinical Trial:	03/05/2021

Abstract

Study Summary

A study was performed at **PacGenomics Clinical Genetics laboratory** to evaluate the test kits performance from 2/26/2021-3/5/2021. Samples obtained from travelers including European travelers were used for the study. All samples are blinded and randomly mixed that the operator does not have prior bias about the result. A total of 412 samples obtained from travelers including 203 European travelers were tested during the study. The samples were tested with the detection reagent and the reference reagent respectively, and the detection results of the two products were compared. Perform statistical analysis on the test results calculate the percentage of diagnostic specificity and sensitivity to the total coincidence rate. According to the statistical analysis results, the applicability and accuracy of the Test Kit are judged, so as to determine whether the test results of the Test Kit are consistent with the test results of the control Kit.

The results of Anbio Rapid COVID-19 Antigen Test (Colloidal Gold) / Nasal Swab showed a good agreement with PCR and clinical results. The clinical Sensitivity of Anbio Rapid COVID-19 Antigen Test (Colloidal Gold) / Nasal Swab is 99.52%, clinical Specificity is 100%. In conclusion, the **Anbio Rapid COVID-19 Antigen Test (Colloidal Gold) / Nasal Swab** has good clinical performance, and meets the clinical requirement.

Dr. Li is a study Coordinator / Supervisor to supervise the entire study process and witness the whole process was done correctly including sample collection, sample extraction, adding and results reading.

CONTENS

I. Introduction

The COVID-19 antigen rapid test is a colloidal gold immunochromatography test for the qualitative detection of nucleocapsid antigens from SARS-CoV-2 in human nasal swabs. It is suitable for use by people who are suspected of having a COVID-19 disease for users over 18 years of age. Children and adolescents under the age of 18 should only take the test under adult supervision.
The new coronaviruses belong to the genus β-corona viruses. The disease they cause, COVID-19, is an acute infectious disease of the respiratory tract. Currently, people infected with the novel coronavirus are the main source of infection, but asymptomatically infected people can also be infectious. The incubation period is 1 to 14 days, usually 3 to 7 days. The main symptoms are fever, tiredness and a dry cough. In some cases, you experience a blocked or runny nose, sore throat, muscle pain, and diarrhea. The test results relate to the identification of SARS-CoV-2 nucleocapsid antigen. The antigen is generally detectable in upper respiratory tract samples or lower respiratory tract samples during the acute phase of infection. A positive test result suggests SARS-CoV-2 infection, but further testing by a doctor is needed to confirm infection. Negative results do not completely rule out SARS-CoV-2 infection.
Negative results should be confirmed by a doctor, especially if symptoms of COVID-19 are present. People who are unable to understand the instructions for use or who are unable to carry out the test themselves should only carry out the test under supervision and with outside help.
This test is based on gold colloidal immunochromatography. During the test, samples are applied to the test cards. If the sample contains the SARS-CoV-2 antigen, the antigen binds to the SARS-CoV-2 antibody. After the sample is applied to the test strip, the complex moves along the nitrocellulose membrane to the end of the absorbent paper. When passing the test line (line T, coated with another SARS-CoV-2 antibody), the complex of SARS-CoV-2 antibody is bound to the test line and shows a red line. When passing line C, a control antibody is bound so that a red line appears.

II. Purpose of the Test

The objective of this study is to evaluate and validate the performance of SARS-CoV-2 antigen test devices by comparison to a reference method,Polymerase Chain Reaction (PCR) assay at the third-party laboratory. The purpose of the study is verification the diagnostic specificity and diagnostic sensitivity of the in vitro diagnostic medical device: SARS-CoV-2 Antigen Test Kit (Colloidal Gold) (hereinafter referred to as "to-be evaluated reagent") for defined specimen type intended to be used during testing procedure by the end-user. The rapid tests are manufactured by **Anbio (Xiamen) Biotechnology Co., Ltd.**
The comparator method should be one of the more sensitive RT-PCR assays authorized by FDA and CE.

1. Clinical Sample Selection

Samples obtained from travelers including European travelers are used for the study. 212 Positive samples and 200 Negative samples were tested during the study.

(1) Inclusion Criteria:

A. Samples from travelers including European Travelers with traceable patients' information

B. The sample should be with clearly recorded source, all samples should have corresponding basic clinical information, including: sample number, gender, age and clinical diagnosis information. The clinical diagnosis information includes asymptomatic or with suspected symptoms of SARS-CoV-2. The collection and treatment of samples are in accordance with the reagent specification or relevant regulations.

(2) Exclusion Criteria:

If the following conditions occur, the test samples will be directly excluded/rejected:

A. Samples with human factors that cannot complete the test process (such as samples that are contaminated during the test operation).

B. The test sample contaminated with bacteria or/and nosebleeds.

C. The test sample gone through too many freeze-thaw cycles during storage.

D. The test sample not kept under the conditions required for storage or testing.

E. Test samples cannot generate control signals by use PCR reagents .

F. Samples without clear medical information

G. Samples without traceable patient information

(3) Quantity of Samples:

212 Positive samples and 200 Negative samples were used during the study to validate the Antigen test results with RT-PCR results.

2. Intended Use

The COVID-19 antigen rapid test is a colloidal gold immunochromatography test for the qualitative detection of nucleocapsid antigens from SARS-CoV-2 in human nasal swab samples. It is suitable to use on people who are suspected of having a COVID-19 disease. Suitable for users over 18 years of age. Children and adolescents under the age of 18 should only take the test under adult supervision.

The new coronaviruses belong to the genus β-corona viruses. The disease they cause, COVID-19, is an acute infectious disease of the respiratory tract. Currently, people infected with the novel coronavirus are the main source of infection, but asymptomatically infected people can also be infectious. The incubation period is 1 to 14 days, usually 3 to 7 days.

The major symptoms are fever, tiredness and a dry cough. In some cases, you experience a blocked or runny nose, sore throat, muscle pain, and diarrhea.

The test results relate to the identification of SARS-CoV-2 nucleocapsid antigen. The antigen is generally detectable in upper respiratory tract samples or lower respiratory tract samples during the acute phase of infection. A positive test result suggests SARS-CoV-2 infection, but further testing by a doctor is needed to confirm infection. Negative results do not completely rule out SARS-CoV-2 infection. Negative results should be confirmed by a doctor, especially if symptoms of COVID-19 are present.

People who are unable to understand the instructions for use or who are unable to carry out the test themselves should only carry out the test under supervision and with outside help.

3. Principle of the Product Test

The double antibody sandwich method is adopted for this product to implement determination in the form of solid phase immunochromatography. The sample to be tested diffuses upward by capillary force at the sampling end, and when passing by the marker pad, the SARS-CoV-2 antigen in the sample is combined with the antibody on the marker pad to form a colloidal gold antibody-antigen complex. The complex continues to spread with the sample to reach the nitrocellulose membrane and is intercepted by the T- line (test line) coated with antibody, and the complex is captured to form an immune complex of colloidal gold antibody conjugates-antigen-coating antibody. The remaining colloidal gold conjugates continue to ascend and are combined with C-line (quality control line), indicating completion of the reaction.

4. Product Performance Parameters

Item No.	Parameters	Standard
1	Clinical Performance	**Sensitivity** 99.52% (95% CI:97.40%~99.99%) **Specificity** 100% (95% CI: 88.78%~100%) **Accuracy** 99.75% (95% CI:98.65%~99.99%)
2	Limit of Detection	150 $TCID_{50}$/ml. Limit of Detection (LoD)
3	Hook Effect	No high dose hook effect was observed when tested with up to a concentration of 1.0 x $10^5 TCID_{50}$/mL of Gamma-inactivated SARS-CoV-2 virus with the Anbio Rapid COVID-19 Antigen Test (Colloidal Gold).
4	Cross reaction	No cross-activity or interference was seen.
5	Interference Response	No cross-activity or interference was seen.

5. Material

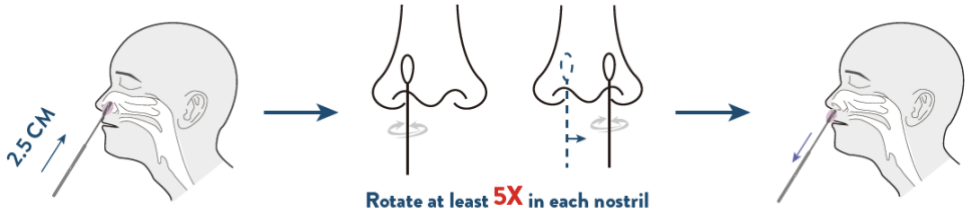
The test kit consists of Test Cassette, Extraction Tube (with Extraction Solution), Sample eluent, and Instructions for Use.

(1) The Test Cassette consists of the card housing and test strip. Test strip contains a sample pad, glass fiber (Colloidal gold labeled anti-SARS-CoV-2 monoclonal antibody), nitrocellulose (NC) membrane, The T-line (test line) of nitrocellulose membrane is coated with mouse anti-SARS-CoV-2 monoclonal antibody, the C-line (quality control line) is coated with internal reference protein C, absorbent paper and PVC plate.
(2) Sample eluent: the main component is phosphate buffer (PBS).
(3) Disposable swab: It is used for sample collection and transfer.

6. Test Procedure

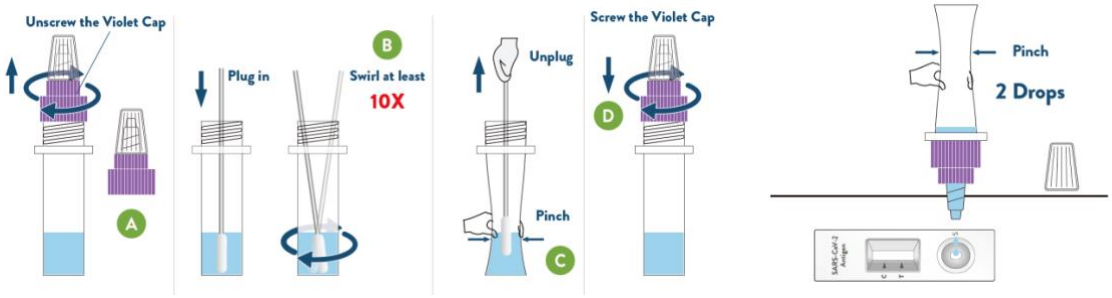
Sample collecting

(1) Make sure the nasal cavity is moist.
(2) The tip of the swab should be inserted between 2 and 2.5 cm until resistance is felt.
(3) Roll the swab along the inner wall of the nostril 5 times to ensure that mucus and cells are collected.
(4) Using the same swab, repeat this process for the other nostril to ensure that a feasible sample is taken from both nostrils. Remove the swab from the nasal cavity.

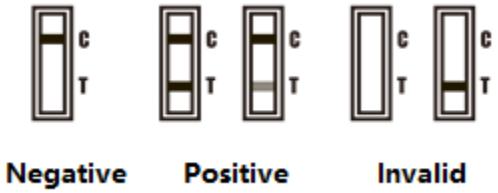


Treatment of Specimen

(1) Remove the white cap from extraction reagent tube.
(2) Insert the sample swab into the tube (immerse the sample part in the elution buffer), make sure the sample is removed into the buffer by rubbing and stirring the sampled swab up & down for 10-15 times.
(3) Squeeze the tube and the swab to leave the eluent on the swab completely in the elution tube.
(4) Discard the swab. Place the dropper tip on the tube.
(5) Mix the sample by gently turning the tube upside down, squeeze the tube to add 2 drops (about 80 μl) to the sample well of the reagent card, and start counting.
(6) Visually read the result after 10 minutes. The result is invalid after 20 minutes.



Interpretation of Results



(This picture is for reference only, and the real product should prevail)

(1) Negative (-):
Only line C is colored (see figure below), which indicates that the sample does not contain any SARS-CoV-2 antigen.

(2) Positive (+):
Colorings can be seen on both line C and line T (see figure below), which indicates that the sample contains SARS-CoV-2 antigen.

(3) Invalid:
No coloration can be seen on line C (see figure below). The test is invalid or an application error has occurred. Repeat the test with a new test cassette.

III. Test Management

1. Overview of Management Structure

This clinical trial was conducted by **PacGenomics Clinical Genetics laboratory**. As the applicant, **Anbio (Xiamen) Biotechnology Co., Ltd.** was responsible for communication and contact during the clinical trial.

2. Quality Control of the Laboratory

(1) All investigators participating in this clinical trial passed the qualification training and had professional background and capacity related to clinical trial. Before clinical trial, all investigators had enough understanding and knowledge for specific contents of the clinical trial protocol and all indicators through training.

(2) The quality control of the laboratory was fully regulated under the CLIA and CAP. And the laboratory met the requirements of quality control of clinical laboratory to ensure the standardization of experimental operating instructions.

(3) Quality control before the analysis: Check whether sample collection and processing meet the requirements, and whether sample number and other information are correct.

(4) Regularly inspect the implementation and completion of the clinical trial. Check integrity and accuracy of clinical sample information and verify test results.

3. Statistics and Data Management

(1) All selected cases were filled in clinical outcome summary, including subject sample number, age, gender, etc. Experimenters filled the test results of both the Reference Kit and the test kit in the clinical outcome summary.
(2) After finishing data entry, main investigators, experimenters and applicant reviewed the data together and locked data without any doubt.
(3) The clinical outcome summary was then submitted to analysts for statistics and analysis. The obtained statics and analysis results were filled in corresponding parts of clinical report.

4. Data Preservation

The test unit and the applicant kept one copy of clinical trial data respectively, including the following: Clinical Test Agreement, Clinical Test Protocol, Ethics Committee Instructions, Clinical Test Report (Test unit reports), General Report on Clinical Test, and Clinical Outcome Summary.

5. Problems Found During Investigation and Measures

In clinical trials, when a small number of samples are tested, the results of the Reference Kit and the Test Kit may be inconsistent. In this case, the clinical quantitative data of the samples involved in the test and the other common clinical trial reagents produced with the same principle are used for re-test.

6. Ethics

PacGenomics Clinical Genetics laboratory will uphold the highest level of ethical practice by respecting potential and enrolled subjects at all times. This includes:

(1) Highlighting all potential risks and benefits of participating in the study prior to patient enrollment and obtaining informed consent.
(2) Respecting participant privacy and keeping their personal information confidential.
(3) Respecting the participant's right to withdraw from the study without penalty.
(4) Answering any questions and addressing any concerns regarding the study, and
(5) Alerting the participant if results from comparator method are abnormal.

7. Financing and Insurance Financing

The financial aspects of this study are documented separately in a confidential agreement between Anbio and principal investigators.

IV. Test Design

1. Description of overall test design and protocol

A total of 412 samples obtained from travelers including 203 European travelers were tested in the study. All samples are confirmed by a EUA approved test with high sensitivity and reverse transcription polymerase chain reaction (RT-PCR). PCR from the FDA SARS-CoV-2 Reference Panel available will

be preferred as a comparator method. Additional information was provided with the samples including: the date of onset of symptoms; the date of sample testing positive or negative by PCR. the SARS-CoV-2 molecular testing details, including specific type of test used and relevant medical history. The specimen should be used to test immediately after collection and should not be frozen and thawed. A blind and randomized method are used for comparison with reliable. All samples are masked with sample ID and randomly mixed. The operators performing Antigen Test are different from the operators performing RT-PCR to ensure pervious bias is not introduced.

2. Specimen Information

Specimen Type:	Nasal Swab
Collection Site:	Hollywood Hills Women's Medical Group
Collection Date:	2/26/2021-3/5/2021
Site Address：	9201 W Sunset Blvd, Suite 401, West Hollywood, CA 90069
QTY. of Collections：	412
Collector:	Dr. Karen Lapesarde

3. Verified Device Information

Product Name:	Rapid COVID-19 Antigen Test (Colloidal Gold) / Nasal Swab
Product Ref. No.:	A6061206
Packing Specification:	20 Tests/Kit
Product Lot No.:	2020116131
Production Date:	2020.11.09
Expiration Date:	2022.11.08
IFU Version:	Rev V1.0
Manufacturer:	Anbio (Xiamen) Biotechnology Co., Ltd.
Address:	No.2016, Wengjiao West Road, Xinyang Street, Haicang District, Xiamen, Fujian, China
Tel	+86 592 6312399
Email:	info@anbio.com

4. RT-PCR Device Information for Control Test

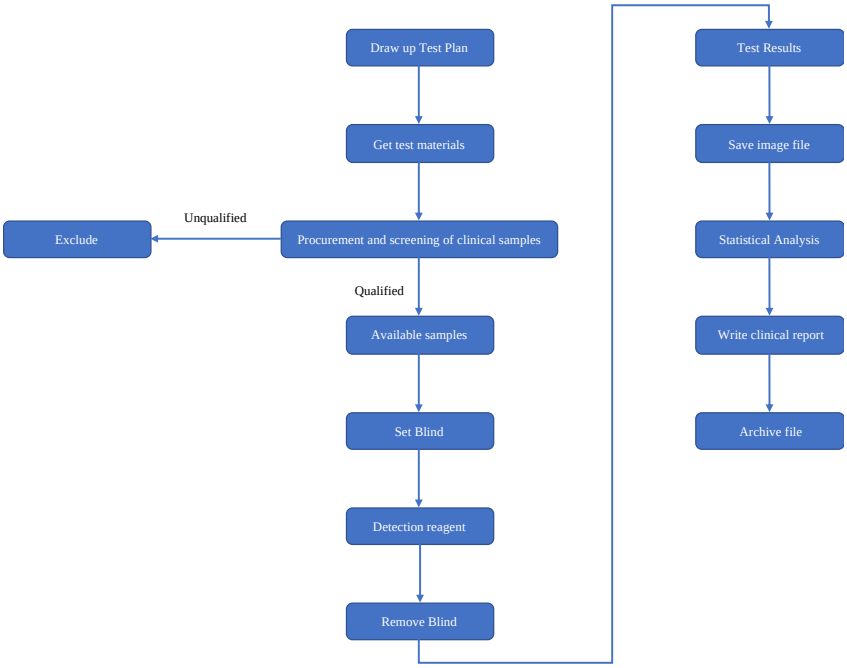
RT-PCR Kit:	OPTI SARS-COV-2 RT PCR
Product Ref. No.:	99-57004
Packing Specification:	500kits/box
Product Lot No.:	20826
Production Date:	8/21/2020
Expiration Date:	7/8/2021
Certification:	CE/FDA-EUA
Manufacturer:	OPTI Medical Systems, Inc.
Address:	235 Hembree Park Drive Roswell, GA 30076 USA
Extraction Kits:	Applied Biosystems MagMax Viral/Pathogen
Product Ref. No.:	A42359
Packing Specification:	550ml/Bottle
Product Lot No.:	2010112
Production Date:	10/11/2020
Expiration Date:	10/11/2021
Certification:	CE, FDA-EUA
Manufacturer:	Thermo Fisher Scientific
Address:	2130 Woodward St, Austin, TX 78744
PCR Analyze:	Applied Biosystems 7500 Fast Dx Real-Time PCR System
Manufacturer	Thermo Fisher Scientific

Address:	2130 Woodward St, Austin, TX 78744
Serial Number of PCR Analyzer:	275032390
Calibration Date:	01/18/2021
Maintenance Due Date:	01/18/2022

5. Laboratory Site Information

Testing Site:	PacGenomics Clinical Genetics laboratory
Registration No:	NPI1841715471
CLIA ID No.	05D2047289
Address:	28222 Agoura Road, Suite 200/201, Agoura Hills, CA 91301, USA
Tel	+1 818-597-1938
Email:	shuanghou@pacgenomics.com
Investigator:	Dr. Hua Li
Audit:	Dr. Lian Liu

6. Test Procedure



7. Data Collection

All the testing data will be listed in datasheet contain matched candidate and reference testing results. (Raw data will be provided for the manufacturer and attached to the final reports as supportive evidence).

8. Statistical Analysis Method of Clinical Evaluation Data

Use the following formula for statistical analysis:

Rapid COVID-19 Antigen Test (Colloidal Gold) / Nasal Swab Results	**RT-PCR Results**		
	Positive	**Negative**	**Total**
Positive	A	B	A+B
Negative	C	D	C+D
Total	A+C	B+D	A+B+C+D
Positive agreement (calculated diagnostic sensitivity)		[A /(A+C)] ×100%	
Negative agreement (calculated diagnostic specificity)		[D/(B+D)] ×100%	
Overall accuracy		[(A+D)/(A+B+C+D)] ×100%	
95% CI		Normal approximation	

V. Result Reading and Interpretation

According to the Instruction for use interoperated, RDT ag results were read after 15 minutes. Any shade in T line is considered to be the positive. Results were also confirmed with the one resulted from the Real-time PCR testing.

Total 412 samples were tested. 1 sample were tested Negative by using Antigen test kits, while the PCR tests were Positive. Please check the following summary table:

Rapid COVID-19 Antigen Test (Colloidal Gold) / Nasal Swab Results	**RT-PCR Results**		
	Positive	**Negative**	**Total**
Positive	211	0	211
Negative	1	200	201
Total	212	200	412
Sensitivity	99.52% (95% CI:97.40%~99.99%)		
Specificity	100% (95% CI: 88.78%~100%)		
Accuracy	99.75% (95% CI:98.65%~99.99%)		

Additionally, sensitivity (positive coincidence rate) withing following CT range representing different viral load will be presented and analyzed. Data should be analyzed separately for each indicated specimen type.

CT range	**CT ≤ 25**	**25 < CT ≤ 28**	**28< CT ≤31**	**31 < CT**	**All CT**
No. of cases (RT PCR)	101	38	34	39	212
No. of positive candidate test results	101	38	34	38	211
Positive coincidence rate	100%	100%	100%	97.44%	99.52%

VI. Conclusion

The results of **Anbio Rapid COVID-19 Antigen Test (Colloidal Gold) / Nasal Swab** showed a good agreement with PCR and clinical results. The clinical sensitivity of Anbio Rapid COVID-19 Antigen Test is 99.52%, clinical specificity is 100%. In conclusion, the Anbio Rapid COVID-19 Antigen Test indicates good clinical performance, and meets the clinical requirement.

Authorization Review

CEO of Pacgenomics: Dr. Lian Liu

Medical Director of Pacgenomics: Dr. Hua Li

About PacGenomics Clinical Genetics Laboratory

PacGenomics, a fully accredited clinical genetics laboratory, we use our ingenuity to create assays that generate the highest resolution and clinical sensitivity. Our innovative scientists are continually working to improve the field of reproductive genetic testing by providing high-quality NGS based testing with excellent client care and support to match. PacGenomics offers Viral PCR Testing and Antibody Testing for Covid-19.

Headquarters' location:

PacGenomics.

CLIA ID No.: 05D2047289

28222 Agoura Road, Suite 200/201, Agoura Hills, CA 91301, USA

Office Phone: +1 818-597-1938

Office Fax: +1 818-597 1939

Institution and Researchers Involved in Clinical Trials

Institution: PacGenomics Clinical Genetics laboratory			
Item.	**Researchers**	**Title**	**Responsibility**
1	Dr. Lian Liu	CEO	Manage and Maintain the qualification of Laboratory
2	Dr. Hua Li	Medical Director	Manage and supervise the clinical study
Institution: Hollywood Hills Women's Medical Group			
3	Dr.Karen Lapesarde	Medical Doctor	Collect samples

Appendix

Appendix A: Laboratory Certificate

Appendix B: Initial Approval Letter

Appendix C: Summary of Clinical Trial Test Results

Appendix D: Image of Verified Device

Appendix E: Image of Control Device

Appendix F: Image of Test Results